Use of Paper Chromatography for the Quantitative Estimation of Quercetin in Rutin*

By J. NAGHSKI,† C. S. FENSKE, Jr., and J. F. COUCH‡

The rapid mobility of quercetin during chromatography on paper forms the basis of its separation and isolation from rutin preparations. The quercetin in the eluate is determined spectrophotometrically after reaction with aluminum chloride. The developing solvent can be modified to prevent interference by related flavonol compounds.

RUTIN (3,5,7,3′,4′-pentahydroxyflavone-3-rutinoside), unless purified by special techniques, contains varying amounts of quercetin (3,5,7,3′,4′-pentahydroxyflavone). Undoubtedly, some of the quercetin is formed by the hydrolysis of the glycoside during processing and some is extracted from the plant. Since quercetin probably has the same physiological activity as rutin, it would not be objectionable, yet its content does reflect the care exercised in the preparation of rutin. The National Formulary (1) specifies a 5 per cent limit on the quercetin content of rutin preparations. The similarity in structure of the two compounds makes it difficult to determine one in the presence of the other.

In 1947 Porter, Brice, Copley, and Couch (8) reported a spectrophotometric method for the simultaneous determination of rutin and quercetin; in 1949 a modification of the method was proposed by Swann (10). Both procedures require specialized apparatus and unusual care in standardization of wave length and preparation of sample, and both are subject to interference from related and extraneous impurities. Porter, Dickel, and Couch (9) developed a method for determination of small quantities of rutin in urine based on production of color with aluminum chloride. Later Dechene (5) adapted this reaction to determination of rutin in tablets. Neither of these procedures is specific for rutin, since other flavonols give the same reaction.

The development by Consden, Gordon, and Martin (3) of partition on a filter paper support gave a powerful tool for the fractionation of difficult mixtures. Since then the method has been applied not only to the separation but also to the quantitative determination of an ever increasing number of compounds. Use of paper chromatography for the separation and identifica-

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‡ Deceased.

tion of flavonoid pigments has been reported by Bate-Smith and Westall (2) and by Wender and co-workers (6, 11). For a quantitative determination, Gage and Wender (6) eluted the flavonol-3-glycosides from the chromatograms with aluminum chloride and measured the intensity of the resulting colored complex with a spectrophotometer. The aglycone, quercetin, however, could not be eluted quantitatively because of adsorption of the complex by the cellulose.

It is possible to approximate the quantity of many compounds by measuring the size and intensity of the spot. Quercetin, however, tails excessively, making it impossible to judge the size or intensity of the spot with any degree of accuracy.

The technique described here depends on the fact that when the chromatograms are developed with ethyl acetate saturated with water, rutin moves slowly (R_F, 0.05), whereas quercetin travels rapidly (R_F, 0.90). By using a short strip of paper, the quercetin runs completely off the bottom while the rutin remains on the paper strip. The quercetin is determined by an adaptation of the method of Porter, Dickel, and Couch (9).

EXPERIMENTAL

Reagents

(*a*) Chromatographic solvents: either (*a*) ethyl acetate saturated with water; or (*b*) a mixture of ethyl acetate, benzene, and water (35:15:50).

(*b*) Aluminum chloride, 0.1 *M* aqueous solution.

(*c*) Potassium acetate, 1 *N* aqueous solution.

(*d*) Methanol or isopropanol.

Apparatus

(*a*) Filter paper, Whatman No. 1[1] cut as illustrated in Fig. 1.

(*b*) Micropipette graduated in 0.01 ml. and fitted with a 26-gauge hypodermic needle.

(*c*) Receivers, 6-oz. bottles cut off as illustrated in Fig. 1.

(*d*) Tank, a 10-in. desiccator fitted with a tray and receivers as shown in Fig. 2. A Pyrex desiccator without a continuous shelf can be adapted by using a perforated plate and a shorter pan support.

(*e*) Spectrophotometer or filter photometer with cell compartment for 2.5-cm. cells.

PROCEDURE

Standard Rutin Preparation.—Rutin was made free of contaminating flavonoid pigments by repeated recrystallization alternately from absolute (98–100%) ethanol and water (4).

Standard Quercetin Preparation.—The purified rutin was hydrolyzed by refluxing for two hours in 2% sulfuric acid. The quercetin was further purified

[1] The mention of commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

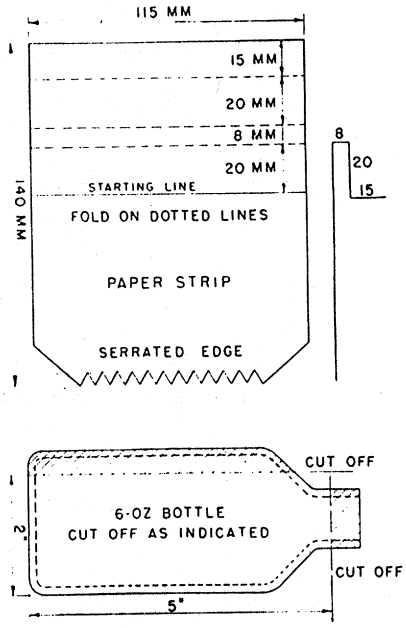

Fig. 1.—Diagram of paper strips and receivers used for the chromatographic elution of quercetin.

by recrystallization from 70–80% ethanol (7). The spectral absorption curves for the quercetin-aluminum complex and pure quercetin are shown in Fig. 3. The absorptivity (a) = A/bc, where A is the absorbance (optical density) of solution of depth, b cm. and concentration, c grams per liter minus the absorbance of an equal depth of solvent.

Separation and Isolation of Quercetin from Rutin.—A 1-Gm. sample of rutin is dissolved in 50 ml. of either methanol or isopropanol. With a micropipette, 0.05 ml. of this solution is applied to the starting line of the filter paper and distributed so that six or seven discrete spots of approximately equal concentration are obtained. By applying small droplets, the size of the spots is kept less than 1 cm. in diameter.

The filter papers are folded and suspended from the tray so that the serrated tips hang within the receivers. Since four pieces of paper can be accommodated at one time, it is possible to run the sample in triplicate with a plain sheet for a blank. The aqueous phase of the solvent is introduced into the

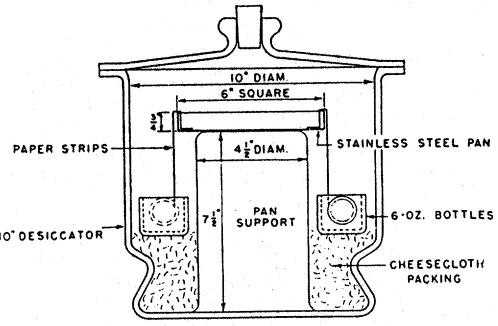

Fig. 2.—Arrangement of pan, paper strips, and receivers in chromatography apparatus.

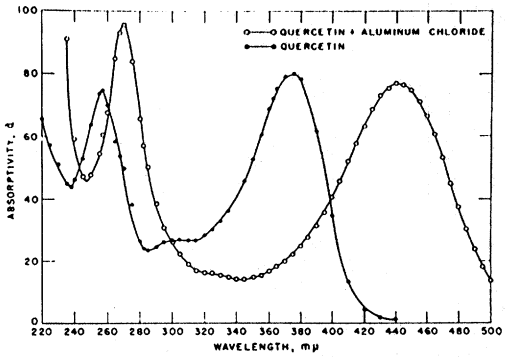

Fig. 3.—Spectral absorption curves for quercetin in 95% ethanol and for quercetin + aluminum chloride in potassium acetate buffered aqueous ethanol.

bottom of the desiccator, and the papers are allowed to equilibrate with the atmosphere for two to three hours. The water-saturated ethyl acetate is then added to the tray, and the chromatograms are developed for sixteen to eighteen hours. During this time, the rutin front moves about 2 to 3 in. down the chromatogram; the quercetin drips off the paper and appears in the eluate.

Spectrophotometric Determination.—The eluates containing the quercetin are transferred from the receivers to 100-ml. beakers by rinsing with alcohol. The solvent is evaporated on a steam bath. To each beaker is added 10 ml. of absolute alcohol, and 3 ml. of 0.1 *M* aluminum chloride solution. The resulting solutions are transferred to 25-ml. volumetric flasks, the beakers are rinsed with 5 ml. of 1 *N* potassium acetate solution, and the solutions are then made to volume with alcohol. After about twenty minutes and not later than one hour, the absorbance of each solution is determined at 440 mμ in a 2.5-cm. cell *versus* a solution in an equal cell prepared from a paper blank carried through the same procedure. After a longer time, the solutions become turbid because of formation of aluminum hydroxide. If excessive interference is caused by too rapid formation of turbidity, the amount of potassium acetate used can be reduced appreciably without affecting the determination, provided the same proportions of reagents are used in the preparation of a standard curve.

Preparation of a Standard Curve.—Pipette a volume of each standard solution, corresponding to 0.030, 0.045, 0.060, 0.075, 0.090, 0.105, 0.120, 0.135, and 0.150 mg. of quercetin per 10 ml. of alcohol, into 25-ml. volumetric flasks, add 3 ml. of 0.1 *M* $AlCl_3$ solution and 5 ml. of 1 *N* potassium acetate solution and make to volume with alcohol. The absorbance as measured against a reagent blank is plotted *versus* weight of aglycone taken for analysis. Table I shows the data obtained in the standardization. A straight line, passing through the origin, was obtained in the range of 0.03 to 0.15 mg. of quercetin.

Recovery Experiments.—In trials using quercetin alone, only 85–90% of the quercetin could be recovered. When rutin was present, however, the recovery was practically complete. This increase in recovery was undoubtedly due to displacement of quercetin by rutin during development of the chromatogram. Synthetic mixtures of rutin containing 4%, 5%, and 6% of quercetin were prepared and chromatographed. The recovery of the quercetin in the eluate is shown in Table II.

TABLE I.—STANDARDIZATION DATA. QUERCETIN-ALUMINUM COMPLEX

Wt. of Quercetin (C^a), Mg.	Absorbance (A^b)	Absorptivity (a)
0.030	0.238	79.3
0.045	0.354	78.7
0.060	0.476	79.3
0.075	0.596	79.5
0.090	0.705	78.3
0.105	0.820	78.1
0.120	0.935	77.9
0.135	1.046	77.5
0.150	1.161	77.4

[a] C = mg. of quercetin in final 25 ml.
[b] A = absorbance at 440 mμ measured on a spectrophotometer in a 2.5-cm. cell with reference to an equal cell containing reagents.

TABLE II.—RECOVERY OF QUERCETIN FROM SYNTHETIC MIXTURES OF QUERCETIN AND RUTIN

Wt. of Quercetin Applied, Mg.	Wt. of Quercetin Determined, Mg.	Recovery %
0.027	0.028	103
0.027	0.026	97
0.027	0.027	100
0.034	0.034	100
0.034	0.033	97
0.034	0.031	91
0.041	0.042	102
0.041	0.040	98
0.034[a]	0.031	91
0.034[a]	0.032	94
0.034[a]	0.032	94
		Av. 97

[a] Solvent composition: ethyl acetate, 35; benzene, 15 water, 50.

Detection and Elimination of Interfering Substances.—Rutin prepared from certain plants contains small amounts of flavonoid pigments in addition to quercetin. Since these substances usually migrate down the paper at a faster rate than rutin, they appear in the eluate and are determined as quercetin. It was found that the R_F values of flavonoids could be controlled by adding benzene to the ethyl acetate. The effect of different amounts of benzene on the R_F values of rutin, quercetin, and other flavonols is shown in Table III. Thus the addition of 15 parts of benzene was sufficient to inhibit the migration of the unknown flavonol glycosides without affecting appreciably the migration of

TABLE III.—EFFECT OF SOLVENT COMPOSITION ON R_F VALUES OF FLAVONOLS

	Solvent Composition, Ml.			
Ethyl Acetate	50	40	35	25
Benzene	0	10	15	25
Water	50	50	50	50
	R_F	R_F	R_F	R_F
Rutin	0.05	0.01	0.0	0.0
Flavonol A	0.32	0.20	0.05	0.0
Flavonol B	0.55	0.41	0.10	0.0
Quercetin	0.90	0.85	0.74	0.50

TABLE IV.—ANALYSIS OF RUTIN FOR QUERCETIN CONTENT BY CHROMATOGRAPHIC AND ULTRAVIOLET SPECTROPHOTOMETRIC METHODS

Sample	—Chromatographic, %—				—Ultraviolet Spectrophotometric, %—			
	1	2	3	Av.	1	2	3	Av.
A	3.3	3.4	3.4	3.4	2.6	3.0	2.6	2.7
B	4.1	4.1	4.2	4.1	4.6	3.9	3.9	4.1
C	5.8	5.8	5.6	5.7	5.9	6.9	...	6.4
D	8.5	8.7	8.8	8.7	9.2	9.4	...	9.3
E	5.2	5.0	5.1	5.1	6.3	5.7	...	6.0
F	2.3	2.6	2.6	2.5	0.0	−0.2	...	−0.1
	0.0[a]	0.0	0.01	Trace	...	...	...	...
G	5.6	5.8	5.7	5.7	4.3	4.0	...	4.2
	0.4[a]	0.3	....	0.4	...	...	...	...

[a] Modified solvent composition (ethyl acetate, 35; benzene, 15; water, 50).

quercetin. The use of this solvent did not interfere with the recovery of quercetin when admixed with rutin (Table II).

To detect the presence of the flavonol pigments which will interfere in the determination of quercetin, the rutin preparation is chromatographed by either the descending technique as recommended by Bate-Smith and Westall (2), or the ascending technique as recommended by Wender and Gage (11). The solution of rutin is applied to the starting line with a capillary pipette to give a spot about 1 cm. in diameter and containing 40 to 50 μg. of flavonol. The paper is equilibrated in an atmosphere of the aqueous phase for two hours and then developed with water-saturated ethyl acetate for four to six hours. The time required for the solvent to travel 30 to 40 cm. varies with the room temperature. After developing, the chromatograms are air-dried, sprayed with a solution of aluminum chloride, air-dried again, and examined under ultraviolet light.[2] The interfering flavonol pigments appear as fluorescent zones located between those of rutin and quercetin. This procedure is extremely sensitive, detecting as little as 0.2 μg. of quercetin. Spraying with potassium acetate as suggested by Dechene (5) quenches the fluorescence and reduces the sensitivity to 1 μg. of quercetin.

Application of Method to Rutin Preparations.—To establish the applicability of this method to the analysis of actual rutin samples, the results (Table IV) were compared with those obtained by the spectrophotometric method of Porter, Brice, Copley, and Couch (8).

DISCUSSION

Results obtained by the ultraviolet spectrophotometric method are in general higher and show greater variation than those obtained by the chromatographic procedure. The higher spectrophotometric values are undoubtedly due to the presence of interfering impurities. The rutin solutions were dark-colored (indicating the presence of absorbing pigments other than rutin and quercetin) and contained different amounts of materials insoluble in alcohol. Considering the inherent expected error by this method of ±0.7 in the percentage of both rutin and quercetin (8), the results obtained by the two procedures are in good agreement.

The chromatographic procedure gave high values with the two samples of buckwheat rutin (F and G). Chromatography of these samples revealed small proportions of flavonols other than rutin and quercetin. Two compounds could be detected with R_F values of 0.32 and 0.55 (Table III). (The characterization of these constituents will be the subject of a later paper.) When the modified solvent was used in the analysis of these samples, it was found that the true quercetin content was low. Thus in sample G the ultraviolet method apparently determined these constituents as quercetin, whereas with sample F an apparently reliable value was obtained, probably through a compensation of errors. The results point to the fact that the chromatographic procedure could be used not only to determine the quercetin but also the content of other minor flavonols in rutin preparations.

[2] Fluorescent black light tubes with purple envelope are satisfactory for this purpose.

SUMMARY

A method has been developed for the specific determination of quercetin in rutin preparations. It is not subject to error due to interfering substances, and is not critical as to standardization of wave length and preparation of sample.

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